

# Blunted Peripheral and Central Responses to Gastric Mechanical and Electrical Stimulations in Diet-induced Obese Rats

Jing Zhang,<sup>1</sup> Weihong Sha,<sup>2</sup> Hongbing Zhu<sup>1</sup> and Jiande D Z Chen<sup>1,2,3\*</sup>

<sup>1</sup>Veterans Research and Education Foundation, VA Medical Center, Oklahoma City, OK, USA; <sup>2</sup>Division of Gastroenterology, University of Texas Medical Branch, Galveston, TX, USA; and <sup>3</sup>Ningbo Pace Translational Medical Research Center, Beilun, Ningbo, China

## Background/Aims

The increase in the prevalence of obesity is attributed to increased food intake and decreased physical activity in addition to genetic factors. Altered gut functions have been reported in obese subjects, whereas, little is known on the possible alterations in brain-gut interactions in obesity. The aim of the study was to explore possible alterations in gastric myoelectrical activity, gastric emptying, autonomic functions and central neuronal responses to gastric stimulations in diet-induced obese rats.

## Methods

Gastric myoelectrical activity, gastric emptying and heart rate variability were recorded in lean and obese rats; extracellular neuronal activity in the ventromedial hypothalamus and its responses to gastric stimulations were also assessed.

## Results

(1) Gastric emptying was significantly accelerated but gastric myoelectrical activity was not altered in obese rats; (2) the normal autonomic responses to feeding were absent in obese rats, suggesting an impairment of postprandial modulation of autonomic functions; and (3) central neuronal responses to gastric stimulations (both balloon distention and electrical stimulation) were blunted in obese rats, suggesting impairment in the brain-gut interaction.

## Conclusions

In diet-induced obese rats, gastric emptying is accelerated, postprandial modulations of autonomic functions is altered and central neuronal responses to gastric stimulations are attenuated. These alterations in peripheral, autonomic and brain-gut interactions may help better understand pathogenesis of obesity and develop novel therapeutic approaches for obesity.

(*J Neurogastroenterol Motil* 2013;19:454-466)

## Key Words

Autonomic function; Brain-gut interaction; Central nervous system; Gastrointestinal motility; Obesity

Received: May 4, 2013 Revised: July 28, 2013 Accepted: August 9, 2013

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

\*Correspondence: Jiande D Z Chen, PhD

Ningbo Pace Translational Medical Research Center, Beilun, Ningbo 315800, China

Tel: +86-574-86818913, Fax: +86-574-86818262, E-mail: [jjandedzchen@gmail.com](mailto:jjandedzchen@gmail.com)

Financial support: This study was supported in part by a grant from the Oklahoma Center for the Advancement of Science and Technology (Grant No. HR07-114).

Conflicts of interest: None.

Author contributions: Jing Zhang, Weihong Sha and Hongbing Zhu conducted experiments, analyzed data and prepared the draft of the manuscript. Jiande D Z Chen designed and supervised the study, and extensively revised the manuscript.

## Introduction

The prevalence of overweight and obesity is commonly assessed by using body mass index (BMI,  $\text{kg}/\text{m}^2$ ) proposed by the National Institutes of Health<sup>1</sup> and the World Health Organization.<sup>2</sup> People with a BMI between 25 and 30 are described as overweight, while those with a BMI over 30 are counted as obese. Currently severe obesity (BMI > 40) affects more than 15 million Americans.<sup>3,4</sup> Obesity has reached epidemic proportions globally, with more than 1 billion adults overweight - at least 300 million of them clinically obese - and it is a major contributor to the global burden of chronic diseases and disabilities. Obesity and overweight pose a major risk for serious diet-related chronic diseases, including type II diabetes, cardiovascular diseases, hypertension, and certain forms of cancers. The health consequences range from increased risk of premature death, to serious chronic conditions that reduce the overall quality of life. The cost for treating obesity and its co-morbidity is over 100 billion in USA alone and is growing significantly.<sup>5</sup>

The development of obesity is attributed to both genetic and environmental factors. Consequently, 2 types of animal models have been widely used in obesity research: genetic and dietary models. Diet is a major factor within current obesogenic environment. It has been well-documented that both the amount and type of dietary fats and carbohydrates can have pronounced effects on body adiposity and parameters of glucose metabolism. The current obesity epidemic has increased interest in rodent models of diet-induced obese (DIO) from basic research. The Sprague-Dawley (S-D) rat model of DIO is reported to exhibit a clear segregation into susceptible and resistant subpopulations shortly after transfer to a high energy diet. Some investigators have demonstrated that adipose from DIO rats and obese humans share a similar global gene-expression pattern and indicated that there was a significant commonality of affected biological pathways between the DIO rat model and human obesity.<sup>6</sup> DIO rats mimic human obesity in increased body weight and adiposity, increased circulating leptin and insulin levels, increased triglyceride levels and decreased insulin sensitivity. All of these suggest that the DIO rat in general does represent an appropriate obesity model. However, little is known on peripheral gastric motility functions, autonomic functions and brain-gut interactions in DIO rats. The stomach acts as a reservoir for the ingested meal in the fundus; transports the food to the body and the antrum, mixes it with gastric secretions, and agitates it for breakdown to small-

er-sized particles, and finally empties the chyme to the duodenum. Gastric motility plays an important role in food consumption and digestion: not only regulating the rate at which nutrients are transported to the small intestine for absorption but also participating in the control of appetite and satiety. Conflicting findings have been reported on the alterations in gastric motility in obese patients.<sup>7</sup> Gastric accommodation, the ratio of postprandial-to-fasting gastric volume, was found to be similar between asymptomatic obese and control subjects.<sup>8,9</sup> Both solid and liquid gastric emptying studies have been performed in obese subjects with conflicting and inconclusive results. In one study, the emptying of liquids in obese subjects was found to be similar to that in healthy controls while solid gastric emptying was accelerated in obese subjects.<sup>10</sup> Enhanced gastric emptying would reduce the negative feedback satiety signal produced by the presence of nutrients inside the stomach, and thus, precipitates a feeling of hunger and shortens the interval between the consecutive meals. In rats, the accelerated gastric emptying was found to be related to overeating and obesity.<sup>11</sup>

In addition to neural and hormonal regulations, gastric motility is also controlled by gastric myoelectrical activity (GMA). GMA consists of slow waves and spike potentials.<sup>12</sup> A non-invasive method similar to electrocardiography, called electrogastrography, has been developed and applied to detect gastric slow waves using abdominal surface electrodes. In adult patients with obesity, gastric slow waves assessed from the electrogastrography were not altered except a decrease in the amplitude of the recording that could be attributed to the thickness of the abdominal wall in obese patients.<sup>13</sup> Similarly, no alterations in GMA were noted in pediatric patients with obesity.<sup>14</sup>

Heart rate variability (HRV) refers to a measure of the beat-to-beat alterations in the heart rate. It is usually calculated by analyzing a time series of beat-to-beat intervals from the electrocardiogram (ECG) or of beat-to-beat intervals derived from an arterial pressure tracing. Recently, HRV has emerged as a simple, non-invasive and established method to evaluate the sympatho-vagal balance at the sinoatrial level. The analysis of HRV is regarded as an indicator of the activity of autonomic regulation of circulatory function and as a marker reflecting the activity of the sympathetic and vagal components of the autonomic nervous system.<sup>15-17</sup> In this study, fasting and postprandial spectral analyses of the HRV signal were performed to compare possible different autonomic functions in control and obese rats.

It is believed that the ventromedial hypothalamus (VMH) is closely related to the regulation of feeding behavior and plays an

important role in the mediation of satiety. It has been well established in our group to study the effects of gut stimulation on neuronal activity changes in the central nervous system.<sup>18,23</sup> One of the previous studies showed that gastric electrical stimulation (GES) activated gastric distention-responsive (GD-R) neurons in the VMH, and the effect was related to the strength of stimulation in normal weight rats.<sup>21</sup> But it is unknown whether the obese rats have the same central neuronal responses to gastric mechanical/electrical stimulation.

Therefore, the aim of the study was to investigate the possible alterations in (1) gastric functions (GMA and gastric emptying), (2) autonomic functions and (3) central neuronal responses to gastric stimulation (balloon distention and electrical stimulation) in DIO rats in comparison with the control lean rats.

## Materials and Methods

### Animals

Fifty male S-D rats at age of 3 weeks were obtained from Charles River Laboratories, USA. The rats were housed in a facility with controlled temperature ( $22 \pm 2^\circ\text{C}$ ) and maintained in 12/12 hours light-dark cycles (light on from 7 am to 7 pm) with water accessible at all the time. The rats were then randomly divided into 2 groups: (1) the control group ( $n = 20$ ), fed with standard laboratory chow, consisting of 10% fat, 60% carbohydrate and 28% protein (Lab Diet Inc, Brentwood, MO, USA); (2) the high-fat (HF) diet group ( $n = 30$ ) fed with HF (condensed milk diet) diet, consisting of 31.8% fat, 51.4% carbohydrate and 16.8% protein (Research Diets Inc, New Brunswick, NJ, USA). Body weight was monitored once every week. At the 15th week, the rats in the HF diet group showing a body weight higher than the highest in the control group were assigned to the DIO group ( $n = 22$ , body weight  $480.0 \pm 8.2$  g in the DIO group vs.  $392.4 \pm 10.8$  g in the control rats). The remaining eight rats in the HF diet group were discarded. The study was approved by the Institutional Animal Care and Use Committees at the University of Texas Medical Branch, Galveston and VA Medical Center in Oklahoma City. All procedures were conducted in accordance with the Guidelines for the American Care and Use of Laboratory Animals.

### Experiment 1: Assessment of Gastric Functions

#### Surgical procedure

After an overnight fast, 10 rats in each group were operated

under anesthesia with an intraperitoneal ketamine hydrochloride (60 mg/kg) and xylazine (8 mg/kg) mixture. Supplemental anesthesia was given throughout the surgery as required. A midline laparotomy was performed and one pair of 28 gauge stainless-steel cardiac pacing wire (A&E Medical, Farmingdale, NJ, USA) was implanted on the serosal surface of the stomach along the greater curvature at 0.5 cm above the pylorus. The distance between the 2 electrodes in the pair was 0.3 cm. The electrodes (naked portion of the pacing wire) penetrated the subserosa and were affixed to the gastric serosa with sutures. The insulated connecting wires were subcutaneously tunneled through the anterior abdominal wall, along the right side of the trunk, and were led outside the skin to the back of the neck for attachment to a bio-signal recorder. The abdominal wall and skin were closed in a simple interrupted pattern.

#### Recording and analysis of gastric myoelectrical activity

GEA was recorded in the conscious state in the above rats (DIO rats,  $n = 10$ ; control rats:  $n = 10$ ) for 30 minutes in the fasting state (overnight fasted) and 30 minutes after a liquid test meal containing phenol red via the chronically implanted serosal electrodes using a physiological recording system (Acqknowledge III, EOG 100A; Biopac System, Inc, Santa Barbara, CA, USA). The GMA signal was displayed on a computer monitor and saved on the PC. The low and high cutoff frequencies of the amplifier were 0.05 and 35 Hz, respectively. For the analysis of gastric slow waves, the signal was further low-pass filtered with a cutoff frequency of 1 Hz and down-sampled at 2 Hz.

Previously validated computerized spectral analyses were performed to derive the percentage of normal gastric slow waves, the dominant frequency (DF) and dominant power of the GMA recordings. (1) The percentage of normal gastric slow waves was the percentage of time during which regular 4-6 cpm slow waves were presented over the 30-minute period. Each recording was divided into blocks of 1 minute without overlapping. The power spectrum of each 1 minute recording was calculated and examined to see if the peak power was within the range of 4-6 cpm. The 1 minute recording was called normal if the peak power was within the 4-6 cpm range. Otherwise it was defined as dysrhythmia. (2) The frequency at which the overall power spectrum of the entire recording displays a peak power in the range of 1.0-9.0 cpm was defined as the DF. The DF has been shown to be equal to the frequency of the gastric slow wave measured from implanted serosal electrodes.<sup>24</sup> Smoothed power spectral analysis was used to produce the overall power spectrum during each recording period, which were 30 minutes in the fasting state and 30

minutes after the test meal. (3) The power at the DF in power spectrum analysis was defined as the dominant power (DP).<sup>25,26</sup> The dominant power of the GMA recording was expressed as decibels (dB).

### Gastric emptying test

The same rats (DIO,  $n = 10$ ; control,  $n = 10$ ) used in the GMA recording were used for the gastric emptying test (after the completion of experiment 2). The animals were fasted overnight and fed with a methylcellulose test meal during the test. Methylcellulose was dispersed in water at 80°C at a final concentration of 1.5% under continuous stirring. The solution was allowed to cool down to 37°C, and was mixed with phenol red (a non absorbable marker). A volume of 1.5 mL of the methylcellulose meal mixed with phenol red (0.5 mg/mL) was given orally into the stomach through a 16-gauge stainless steel feeding needle which was removed immediately after delivery of the solution. Thirty minutes after the injection of the meal, the rats were rapidly euthanized and the stomachs were clamped at the pylorus and the gastroesophageal junction and then removed. The stomachs were then placed in 100 mL of 0.1 N NaOH, cut into small pieces, homogenized for 30 seconds and then the suspension was allowed to settle for 60 minutes at room temperature. Afterwards, 5 mL of supernatant was taken out of the solution and put into a test tube with 0.5 mL of trichloroacetic acid (20% wt/vol), and centrifuged at 3,000 rpm for 30 minutes. The content of the centrifuged tube was then transferred into another test tube and added with 4 mL of 0.5 N NaOH. The absorbance of the sample was read at a wavelength of 560 nm with a spectrophotometer. Gastric retention was calculated based on the amount of phenol red recovered from the stomach 30 minutes after the meal.

### Experiment 2: Assessment of Autonomic Functions

This experiment was also performed in the same 20 rats used in experiment 1 before the gastric emptying test. The autonomic functions were assessed by the spectral analysis of the heart rate variability signal that was derived from the ECG.

The ECG was recorded for 30 minutes in the fasting state and 30 minutes after a liquid test meal containing phenol red. A special amplifier (2283ft/i Fetode Amplifier; UFI, Morro Bay, CA, USA) with a recording range of 1.5 to 100 Hz was used to record the ECG via the pair of electrodes implanted on the gastric serosa and a third electrodes on the skin of the rat body. Typically, the ECG is recorded using abdominal skin electrodes. However, in this rodent study, we found that the quality of the

ECG signal was much better when the gastric serosal electrodes were used. The ECG signal was digitized using the sound card installed on the PC at a sampling frequency of 500 Hz, displayed on a computer monitor, saved on a PC. The HRV signal was derived from the original ECG recording by identifying R waves, interpolating R-R interval data (bilinear interpolation) at 100 Hz, and, finally, down sampling the interpolated HRV data at 8 Hz suitable for spectral analysis.

Spectral analysis of the HRV signal was performed using the smoothed spectral analysis method.<sup>27</sup> Spectral powers at two frequency ranges were averaged using a method published in the literature<sup>28</sup>: (1) a high-frequency band (HF: 0.8 to 4.0 Hz) reflecting parasympathetic or cardiac vagal efferent activity; and (2) a low-frequency band (LF: 0.3 to 0.8 Hz) reflecting mainly sympathetic activity.

### Experiment 3: Central Neuronal Responses to Gastric Distention and Electrical Stimulation

#### Surgery preparation

Ten control rats and 12 DIO rats were used in this experiment. After general anesthesia, a catheter was inserted into the right carotid artery to monitor blood pressure by a pressure transducer. A plastic tube was inserted into the trachea by tracheotomy for artificial ventilation using a volume-control pump (55-60 strokes/min, 3-5 mL stroke volume). After midline laparotomy, the stomach and proximal duodenum were exposed, a small incision was made at the fundus, and the stomach was gently cleaned by a small spoon. A latex balloon was placed in the proximal stomach via incision in the fundus and secured in place by a purse-string suture. One pair of same cardiac pacing wires used in experiment 1 was sutured onto the serosal surface of gastric antrum close to the lesser curvature for delivering electrical stimulation. After the abdominal surgery, the rat was positioned on the stereotaxic frame and the dorsal surface of the brain was exposed. Small holes were drilled in the skull to expose the cortex, and the dura was cut. The stereotaxic coordinates were used in accordance with the atlas of the rat brain.<sup>29</sup> A one-barrel glass microelectrode filled with 0.5 M sodium acetate and 2% pontamine sky blue (tip diameter 3-10  $\mu\text{m}$ , resistance 5-15 M $\Omega$ ) was advanced with the aid of a hydraulic micro-positioner into the area of the VMH (2.3-2.8 mm posterior to the bregma, 0.5-1.0 mm right/left lateral to the midline and 8.8-10.0 mm below the outer surface of the skull). The open part of the brain was covered by 3% agar in saline to limit any displacement due to respiration or

heartbeats.

### Experimental procedures

After the microelectrode was advanced into the area of the VMH, the extracellular action potentials of single neurons were recorded via the glass microelectrode (with the ground electrode placed on the epicranium of the rat, amplified using a high input impedance amplifier (2400A; Dagan corporation, Minneapolis, MN, USA), displayed on an oscilloscope. All data measured with or without GD or GES were recorded and stored in a computer with a sampling frequency of 100 Hz for further analyses.

When a neuron was identified and its firing pattern had become stable for at least 10 seconds, GD was performed by inflating the gastric balloon with air for 20 seconds to determine whether the neuron was responsive to GD. Once a neuron was identified as GD-R neuron, a few more episodes of distention with different pressures (20, 40 and 60 mmHg for 10–20 seconds) were applied in a randomized order with a sufficient recovery period in between 2 consecutive episodes of distention. After the GD test, GES was applied for one minute with using pulse trains (train on-time of 2 seconds, off-time of 3 seconds; pulse width of 0.3 milliseconds; frequency of 40 Hz and amplitude of 6 mA) and the neuronal response to GES was recorded. This set of GES parameters was the same as the one used in clinical studies in which GES was applied for the treatment of obesity.<sup>30–32</sup>

After the recording, a cathodal current (10 mA, 20 minutes) was applied by the same glass microelectrode to mark the recording site. At the completion of the entire study in a rat, the rat was euthanized and the brain was removed and stored in fixative solution. Frozen sections of 40–50  $\mu\text{m}$  of the brain were viewed by a microscopy to identify the recording locations. If the recording sites (lesion generated by the electrical current) were outside of the VMH, the data were excluded from the analysis.

A total of 83 neurons were studied in this way from the 10 control and 12 DIO rats. In average, around 4 neurons were studied in each rat.

### Data analysis

All signals measured with or without GD or GES were recorded and stored in a computer for analyses. Spontaneous activity of neurons was determined by counting activity for 10 seconds and then dividing by 10 to obtain impulses per second. An increase or decrease of 20% from the baseline level of neuronal activity during GD or GES was considered as an excitatory or inhibitory response to GD or GES. A neuron that was responsive to GD of low pressure (20 mmHg) was classified as a low threshold (LT) neuron, whereas a neuron that was responsive only to

GD of high pressure (40 and 60 mmHg) was called a high threshold (HT) neuron. The percentages of neurons responsive to GD/GES in the control and DIO rats were determined.

### Statistical Methods

All the data are presented as mean  $\pm$  SE. Unpaired t test was used to determine the difference in any of the above functions/parameters between the control and DIO rats.  $P < 0.05$  was considered statistically significant.

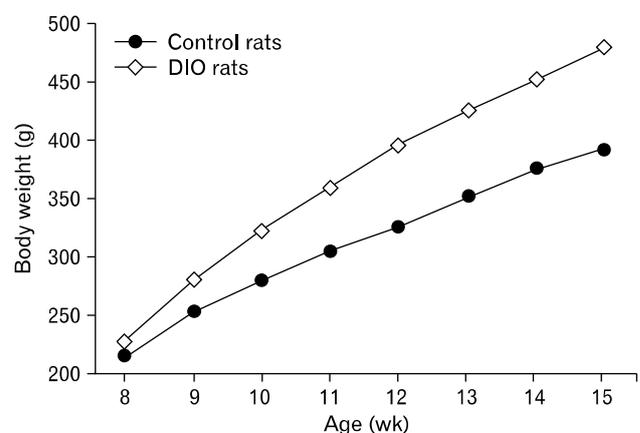
## Results

### Development of Obesity

Figure 1 shows the growth of the rats from the age of 8 weeks to 15 weeks, and the body weight gain during the feeding period is gradual. The diet-induced phenotype becomes most apparent after 8 weeks of HF feeding. The control and DIO rats had similar body weight at the age of 8 weeks. However, at the age of 15 weeks, the DIO rats weighed 21.9% more than the control rats ( $n = 22$ ;  $480.0 \pm 8.2$  g vs.  $392.4 \pm 10.8$  g;  $P < 0.05$ , unpaired t test). About 73% of the male S-D rats fed with the HF diet for 15 weeks in the current study became obese.

### Gastric Motility Functions

Gastric slow waves were not altered in the DIO rats compared with the control rats, as shown in Table 1. The percentage of normal gastric slow waves of DIO rats was  $83.0 \pm 3.3\%$  in the fasting state and  $85.2 \pm 2.7\%$  in the fed state; these were com-

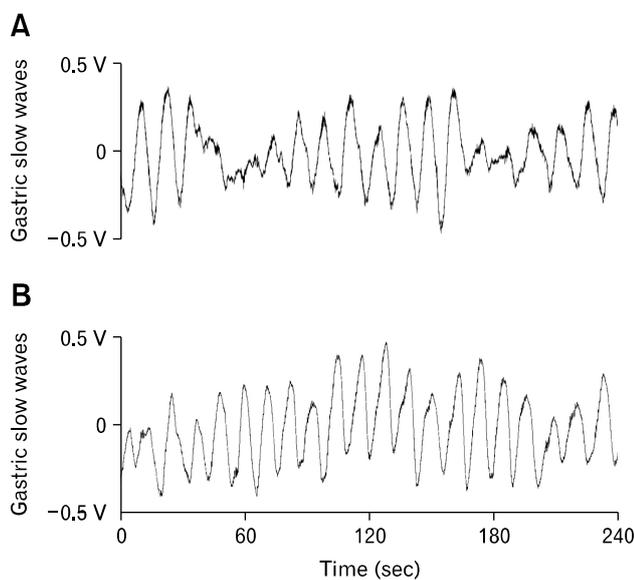


**Figure 1.** Comparison of body weight changes in the control and diet-induced obese (DIO) rats from the age of 8 to 15 weeks.

**Table 1.** Major Electrogastrography Parameters in Control and Diet-induced Obese Rats

	DIO rats		Paired <i>t</i> test	Control rats		Paired <i>t</i> test
	Fasting	Postprandial		Fasting	Postprandial	
DF (cpm)	5.0 ± 0.1	5.6 ± 0.1	< 0.05	5.0 ± 0.2	5.3 ± 0.2	NS
DP (dB)	-2.5 ± 0.8	-4.3 ± 1.0	NS	-5.4 ± 0.9	-4.7 ± 0.8	NS
% of normal slow wave	83.0 ± 3.3	85.2 ± 2.7	NS	87.2 ± 3.3	83.6 ± 1.2	NS
% of bradygastria	3.2 ± 1.3	2.0 ± 1.5	NS	3.5 ± 1.0	3.6 ± 1.4	NS
% of tachygastria	8.6 ± 2.6	9.3 ± 1.2	NS	2.9 ± 2.9	7.1 ± 1.7	NS
% of arrhythmia	5.3 ± 2.4	3.6 ± 2.0	NS	6.4 ± 1.0	5.7 ± 2.2	NS

DIO, diet-induced obese; DF, dominant frequency; DP, dominant power.



**Figure 2.** Regular gastric slow waves were recorded from a control rat (A) and a diet-induced obese rat (B). The frequency of the gastric slow wave was about 5 cycles/minute.

parable with those of the control rats. Figure 2 shows the regular gastric slow waves observed in a DIO rat and a control rat. Similarly, no difference was noted in the percentage of bradygastria, tachygastria or arrhythmia between the DIO and control rats in either fasting or fed state. Although there was a significant postprandial increase in the DF of the slow waves in the DIO rats that was not appreciated in the control rats, no difference was noted in the frequency between the DIO and control rats.

Gastric emptying was significantly accelerated in the DIO rats compared with the control rats. The percentage of gastric emptying at 30 minutes after the meal ingestion was  $76.3 \pm 1.8\%$  in the DIO rats and  $64.9 \pm 3.4\%$  in the control rats ( $P < 0.05$ , unpaired *t* test); an 18% increase was appreciated.

## Autonomic Functions

No difference was noted in the sympathetic and parasympathetic activities derived from the spectral analysis of the HRV signal in the fasting state between the control and DIO rats (Table 2). However, the postprandial alterations observed in the control rats were absent in the DIO rats, suggesting an impairment of postprandial modulation of autonomic functions in the DIO rats. As shown in Table 2, the sympathetic component, LF and the vagal component, HF in the DIO rats of the fasting state were comparable to the corresponding values in the control rats ( $P > 0.05$ , unpaired *t* test). In the control rats, significant changes were noted in LF, HF and LF/HF ratio (the index of the sympathovagal balance) between the fasting and postprandial states, suggesting meal-induced alterations in the autonomic functions. These meal-induced changes were however, absent in the DIO rats, indicative of blunted postprandial changes in the autonomic functions.

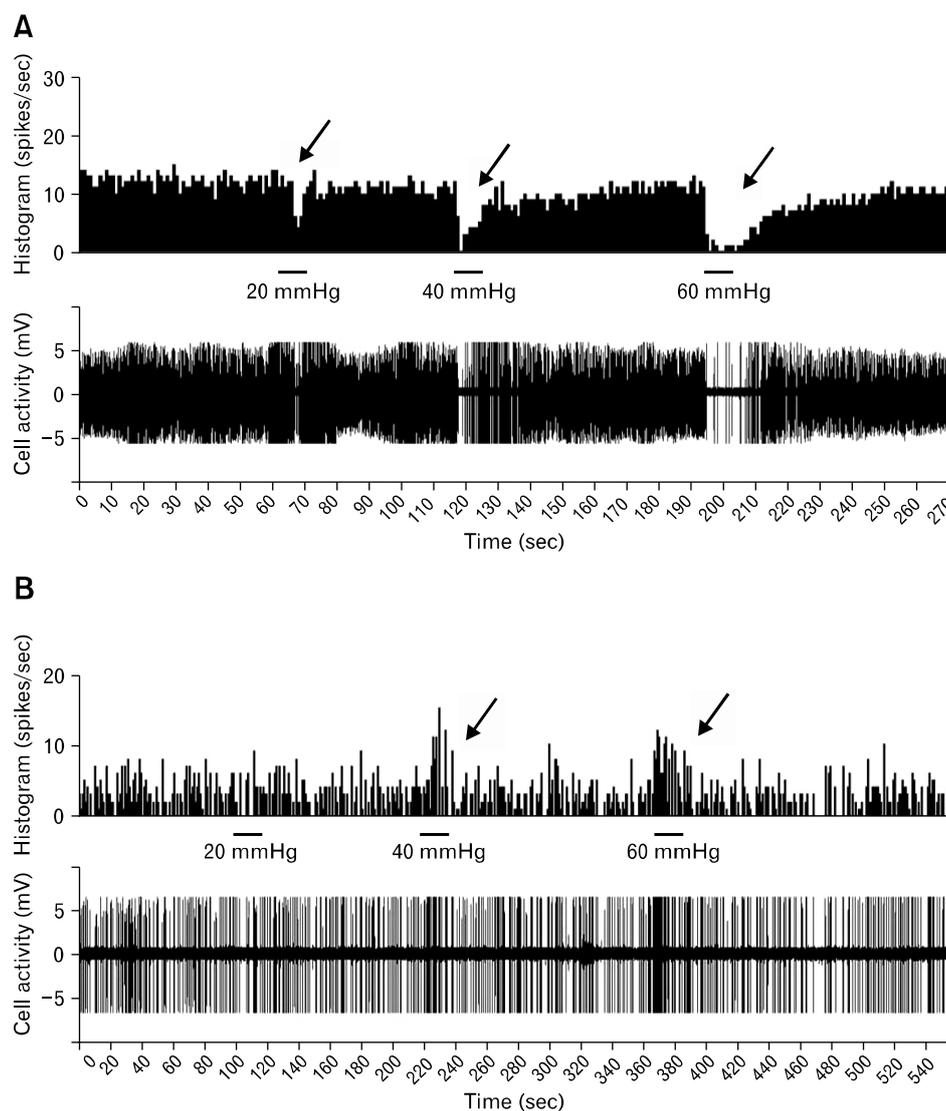
## Central Neuronal Responses to Gastric Stimulation

Sixty-one of the 83 neurons were responsive to GD of up to 60 mmHg while the rest 22 neurons did not respond to GD. The VMH neurons in the DIO rats were found to be less sensitive to GD. A significantly lower percentage of neurons (LT/LT + HT, 12/38, 31.6%) were activated by GD of 20 mmHg (LT) in the DIO rats, compared with that in the control rats (14/23, 60.8%) ( $P = 0.048$ , unpaired *t* test). This finding suggested that the DIO rats were more resistant to GD at a physiological pressure. No significant difference was noted in the percentage of the neuronal responses to GD of 60 mmHg (HT) between the DIO rats and the control rats (69.7% vs. 76.0%;  $P > 0.05$ , unpaired *t* test). Typical tracings are presented in Figure 3, showing the different responses to GD between a DIO rat and a control rat.

**Table 2.** Heart Rate and Autonomic Functions in Control and Diet-induced Obese Rats

	DIO rats		Paired <i>t</i> test	Control rats		Paired <i>t</i> test
	Preprandial	Postprandial		Preprandial	Postprandial	
Heart rate	215.86 ± 6.82	223.57 ± 6.91	NS	210.45 ± 6.21	227.85 ± 5.00	< 0.01
LF/HF	0.64 ± 0.21	0.43 ± 0.10	NS	0.92 ± 0.49	0.41 ± 0.06	< 0.01
LF	0.39 ± 0.05	0.30 ± 0.04	NS	0.48 ± 0.05	0.29 ± 0.03	< 0.05
HF	0.61 ± 0.05	0.70 ± 0.04	NS	0.52 ± 0.05	0.71 ± 0.03	< 0.05

DIO, diet-induced obese; LF, low-frequency band; HF, high-frequency band.



**Figure 3.** Typical tracings (A-D) of various neuronal responses to gastric distention (see arrows). (A) Low threshold inhibitory responses to both non-noxious (20 mmHg) and noxious (40 and 60 mmHg) gastric distention in a control rat. (B) High-threshold excitatory responses only to noxious (40 and 60 mmHg) gastric distention in a control rat. (C) Low threshold excitatory responses to both non-noxious (20 mmHg) and noxious (40 and 60 mmHg) gastric distention in a diet-induced obese rat. (D) High-threshold inhibitory responses only to noxious (40 and 60 mmHg) gastric distention in a DIO rat. In figures A to D: the upper panels show the histograms of the neuronal activity rates and the bottom panels show the original cell activity. Figures C and D are presented at page 461.

Similarly, the VMH neuron responses in the DIO rats were less responsive to GES in comparison to the control rats. The percentage of responses to GES in the DIO rats was significantly lower compared with that in the regular rats (31.5% vs. 78.2%, *P*

< 0.03, unpaired *t* test). Figure 4 shows the effects of GES on VMH GD-responsive neurons in a regular rat and a DIO rat.

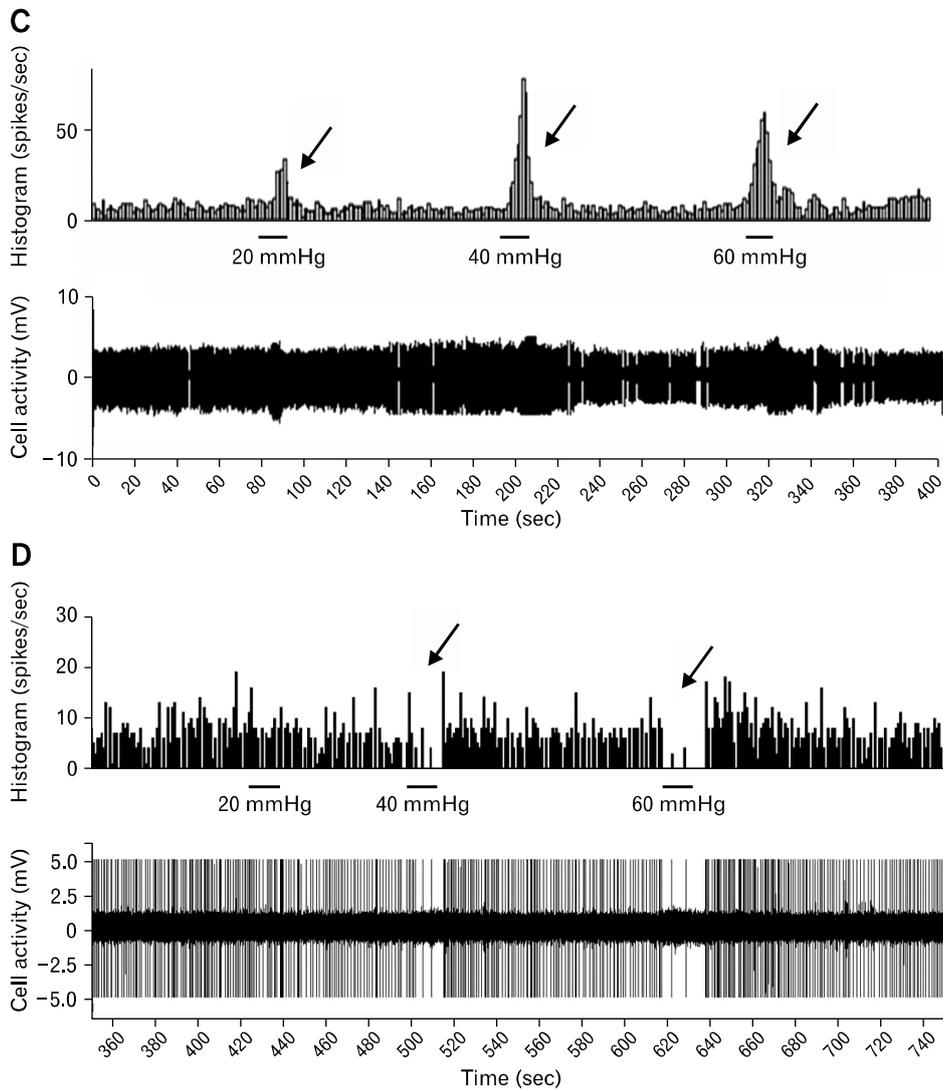


Figure 3. Continued.

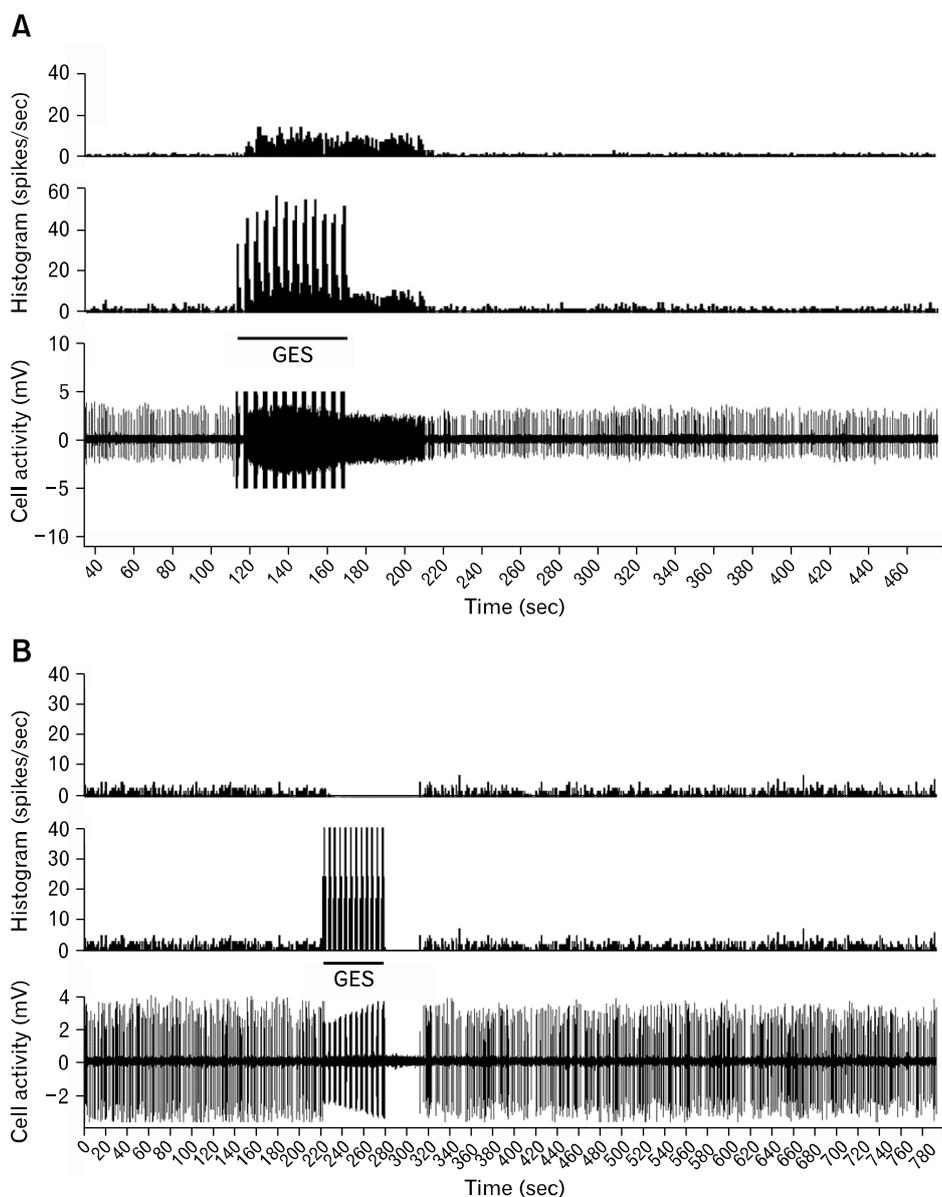
## Discussion

In the present study, there were marked differences in liquid gastric emptying, postprandial gastric slow waves and central neuronal responses to GD and GES between the control rats and the DIO rats.

In general, human obesity is not largely attributed to specific mutations in a particular gene, but represents the outcome of an underlying multi-gene predisposition or susceptibility to obesity.<sup>33</sup> Diet is a major factor in the current obesogenic environment. HF diet is known to induce obesity and metabolic disorders in rodents that resemble the human metabolic syndrome.<sup>34</sup> Obesity can be more effectively induced and last longer when

HF diet is given at a young age.<sup>35</sup> The DIO rat has been reported to show a different energy metabolism with lower energy expenditure and an increased respiratory quotient that indicates a lower use of fat as fuel substrate favoring the fat storage.<sup>36,37</sup> The model of diet-induced obesity in rodents shares a number of similar phenotypes of human obesity, including polygenic inheritance, insulin resistance,<sup>38</sup> hyperlipidemia,<sup>39</sup> hypertension<sup>40</sup> and central leptin resistance.<sup>41-43</sup> Thus, the DIO rodent model is a valuable tool for assessing the underlying biological processes that contribute to the development of obesity in humans.

In the present study, we found that gastric emptying was accelerated in the DIO rats compared with the control ones. It has been reported that obesity is associated with an altered rate of gastric emptying. Green et al<sup>44</sup> observed an acceleration of gastric



**Figure 4.** Effects of gastric electrical stimulation (GES) on gastric distention-responsive (GD-R) neurons in the ventromedial hypothalamus of regular and diet-induced obese (DIO) rats. (A) Excitatory effects of GES on GD-R neuron in a control rat, GES excited the spontaneous neuronal activity compared with baseline (before GES); (B) Inhibitory effects of GES on GD-R neuron in another control rat, GES inhibited the spontaneous neuronal activities compared with baseline; (C) Effects of GES on GD-R neuron in a DIO rat, there is no obvious changes with GES on the spontaneous neuronal activity during and after stimulation compared with baseline. In figures A to C: the upper and middle panels show histograms without and with stimulation artifacts, and the bottom panels show original neuronal activity. GES parameters: train on-time of 2 seconds, off-time of 3 seconds; pulse width of 0.3 milliseconds; frequency of 40 Hz and amplitude of 6 mA. Figure C is presented at page 463.

emptying in obese Zucker diabetic rats of early non-insulin-dependent diabetes mellitus. An earlier study has also showed an acceleration of gastric emptying in rats with obesity resulting from lesions in the VMH.<sup>11</sup> The gastric emptying rate in obese humans has been reported to be controversial because of the variety or the lack of standardization of measurements or methods used.<sup>45</sup> Abnormal gastric emptying was reported in a number of studies in obese patients.<sup>46-48</sup> A study using 300 mL liquid fat-rich meal showed no difference in gastric emptying between obese and lean groups but improved absorption in the upper part of the intestine was observed in obese subjects.<sup>49</sup> Some other studies reported a rapid solid gastric emptying rate in obese pa-

tients than non-obese subjects and the authors suggested that the faster rate of gastric emptying in obese subjects might be a fundamental cause of obesity.<sup>48-50</sup> In another set of studies, gastric emptying of solid and semi-solid was assessed using non-invasive <sup>13</sup>C-octanoic acid and <sup>13</sup>C-acetic breath tests in morbidly obese and non-obese subjects; the data demonstrated a prolonged lag phase and a significantly enhanced gastric emptying of the solid but not semi-solid meal in morbidly obese patients.<sup>51-53</sup> In a most recent study with subjects assigned to consume different isocaloric diets adjusted for BMI and activity level, solid gastric emptying, gastric volume and satiation were found unchanged in obese patients.<sup>54</sup> While these clinical studies revealed incon-

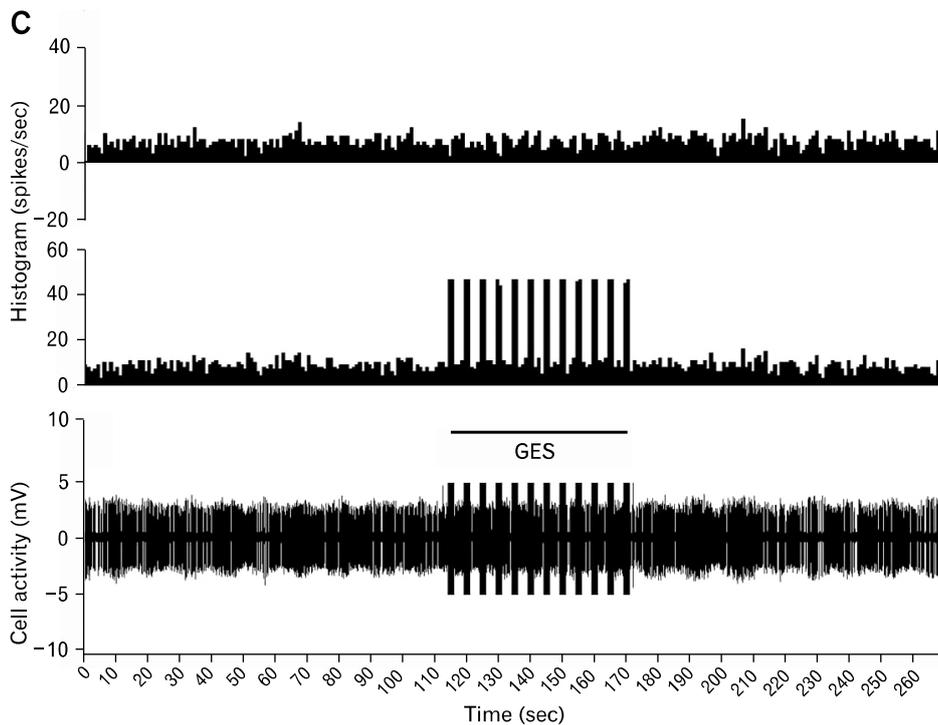


Figure 4. Continued.

clusive data, our finding of the enhanced gastric emptying in the DIO rats is in agreement with the hypothesis that rapid gastric emptying in obese rats increases caloric or food intake due to a more rapid loss of satiety.

An altered postprandial response in autonomic functions to the test meal was found in the DIO rats in the present study according to the spectral analysis of the HRV signal. Although no differences were noted in the sympathetic and parasympathetic activities in the fasting state between the control and DIO rats, the postprandial alterations observed in the control rats were absent in the DIO rats, suggesting an impairment of postprandial modulation of the autonomic functions in the DIO rats. We believe that this impaired response to food intake is important for the development of obesity. A number of previous studies showed altered sympathetic activity in the obese. A study using the 24-hour urinary catecholamine excretion (norepinephrine) as a possible phenotypic marker for sympathetic activity showed higher basal sympathetic activity in DIO-prone rats.<sup>55</sup> DIO rats were also found to have diminished basal and food-related neuronal activation in certain brain areas involving food intake and autonomic function.<sup>56-58</sup> Altered sympathetic activity was noted: sympathetic activity varied markedly as with the function of both dietary composition and relative body weight during the development of DIO.<sup>58,59</sup> The morbidly obese rats with VMH lesion exhibited

low or impaired sympathetic activity.<sup>60-64</sup> The VMH is a site in the hypothalamus for the regulation of the autonomic nervous system response to feeding; rats with damages in the VMH were reported to develop obesity and hyperphagia with reduced sympathetic nervous system activity, delayed satiety and increased parasympathetic activity.<sup>61,62,65</sup> While the sympathetic nervous system activity is highly differentiated in human obesity as published in the literatures, the available data seemed to indicate that obesity was associated with elevated, reduced or no changes in sympathetic activity.<sup>66,67</sup> Data obtained from the analysis of the HRV in obese subjects suggested reduced vagal activity and increased LF/HF ratio in the fasting state in the obese, compared to lean subjects.<sup>68,69</sup>

Attenuated central responses to GD and GES were observed in the DIO rats in this study and these have never been reported elsewhere to the best of our knowledge. The GES parameters we chose in the study were based on the clinical GES studies for obesity treatment. These parameters were later found ineffective in reducing body weight in animals or humans. However, they were effective in altering central neuronal activities and therefore suitable for this study because the goal of this study was to compare the central neuronal responses to GES between lean and DIO rats. The VMH neuronal activity in the DIO rats was found to be less sensitive to GD and GES compared to the control rats. It

is known that in obese and lean animals/human subjects, there are dramatic differences in central metabolism, central pathways, hormonal expressions and functional signaling transit etc. Recently, Dr. Bouret et al<sup>70</sup> used axonal labeling method and showed that selectively bred DIO rats, which are known to be leptin resistant, had defective arcuate nucleus of the hypothalamus (ARH) projections that persisted into adulthood. It is generally accepted that projection pathways from the ARH to other parts of the hypothalamus play a key role in the neural control of food intake and body weight, and leptin is required for normal development of ARH projections. The authors indicated that rats of genetic predisposition toward obesity displayed an abnormal organization of hypothalamic pathways involved in energy homeostasis.<sup>70</sup> Adult DIO rats showed evidence of central leptin resistance.<sup>71-73</sup> The DIO rats were reported to have less leptin-induced phosphorylated signal transducers and activators of transcription 3 (pSTAT3) expression in the hypothalamic arcuate, ventromedial and dorsomedial nuclei.<sup>73</sup> While on low-fat chow diet, rats prone to DIO over expressed arcuate nucleus neuropeptide Y mRNA compared to diet-resistant rats.<sup>74</sup> DIO-prone rats were observed to have reduced sensitivity to the leptin-inhibited effects on the ARH neuropeptide Y expression.<sup>72</sup> These studies demonstrated that selectively bred DIO rats had a defect in central leptin signaling, which antedated the onset of obesity. Binge-eating has been related to a dysfunction in the ghrelin signaling system.<sup>75</sup> Bulimic patients were found to show a lack of postprandial elevation in cerebrospinal fluid glucose concentrations; and it was conjectured that defective transport of glucose across the blood-brain barrier might account for the eating disorders.<sup>76</sup> The neurobiological mechanisms of obesity are not well understood. A recent study used positron emission tomography to assess the neurobiological responses to GES and reported that obese patients with 1-2 year's chronic gastric electrical stimulation showed significant changes in regional brain metabolism; the statistical parametric mapping analysis of the brain metabolic images revealed enhanced activity in certain regions of the brain.<sup>32</sup> Our data shows that obese rats are more resistance to GD with a physiological pressure and gastric electrical stimulation. These blunted reflexive effects from the gastric mechanical and electrical stimulations (GD and GES) to the brain in the obese rats would decrease the feedback satiety signal produced from the stomach. This helps us better understand the central hypothalamic mechanisms of obesity. It should be pointed out that the exact mechanisms involved in the observed blunted responses to gastric mechanical and electrical stimulations in obese rats were

not explored in the present study and deserve future investigations. Intuitively, the blunted central responses to gastric stimulations could be attributed to the adaptation of the stomach to overeating (the stomach is constantly distended more in the obese rats than in the lean animals). From this study, it is clear that an appropriate animal model of obesity is needed in the development of therapeutic approaches for obesity since the peripheral and central responses to food intake and GD in obese are different from those in normal lean subjects. Although it is inconclusive whether patients with diabetes have accelerated gastric emptying, targeting gastric emptying might still be a good method for the development of novel obesity therapy. This is because whether the patient has normal or accelerated gastric emptying, delaying in gastric emptying by any obesity therapy would lead to prolonged meal interval and enhanced postprandial fullness and satiety. Based on the blunted responses of the obese rats to food intake and gastric stimulations (both mechanically and electrically), one can anticipate that the development of therapeutic approaches for obesity is challenging and needs careful design and consideration. As shown in this study, the phenotypes of obese involved both peripheral and central mechanisms as well as the brain-gut axis; it is therefore conceivable that for any therapeutic approaches to be effective, they must involve multiple pathways.

In conclusion, the findings of this study verify the existence of the alterations in gastric emptying, autonomic and central neuronal functions in obese rats. Diet-induced obesity is featured with abnormal or blunted responses to food intake and to gastric mechanical and electrical stimulation. Presumably, these findings not only reveal the possible peripheral and central functional changes in obese rats, but also help design and develop novel treatment methods of obesity.

## References

1. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults - the evidence report. National Institutes of Health. *Obes Res* 1998;6(suppl 2):51S-209S.
2. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser* 2000;894:i-xii, 1-253.
3. Kuczmarski RJ, Carroll MD, Flegal KM, Troiano RP. Varying body mass index cutoff points to describe overweight prevalence among U.S. adults: NHANES III (1988 to 1994). *Obes Res* 1997;5:542-548.
4. Kuczmarski RJ, Flegal KM, Campbell SM, Johnson CL. Increasing prevalence of overweight among US adults. *The National Health and Nutrition Examination Surveys, 1960 to 1991. JAMA* 1994; 272:205-211.

5. Raebel MA, Malone DC, Conner DA, Xu S, Porter JA, Lanty FA. Health services use and health care costs of obese and nonobese individuals. *Arch Intern Med* 2004;164:2135-2140.
6. Li S, Zhang HY, Hu CC, et al. Assessment of diet-induced obese rats as an obesity model by comparative functional genomics. *Obesity (Silver Spring)* 2008;16:811-818.
7. Xing J, Chen JD. Alterations of gastrointestinal motility in obesity. *Obes Res* 2004;12:1723-1732.
8. Kim DY, Camilleri M, Murray JA, Stephens DA, Levine JA, Burton DD. Is there a role for gastric accommodation and satiety in asymptomatic obese people? *Obes Res* 2001;9:655-661.
9. Klatt S, Pieramico O, G uthner C, et al. Proximal gastric motility functions are normal in severe obesity. *Digestion* 1997;58:115-119.
10. French SJ, Murray B, Rumsey RD, Sepple CP, Read NW. Preliminary studies on the gastrointestinal responses to fatty meals in obese people. *Int J Obes Relat Metab Disord* 1993;17:295-300.
11. Duggan JP, Booth DA. Obesity, overeating, and rapid gastric emptying in rats with ventromedial hypothalamic lesions. *Science* 1986;231:609-611.
12. Yin J, Chen JD. Electrogastrography: methodology, validation and applications. *J Neurogastroenterol Motil* 2013;19:5-17.
13. Riezzo G, Pezzolla F, Giorgio I. Effects of age and obesity on fasting gastric electrical activity in man: a cutaneous electrogastrographic study. *Digestion* 1991;50:176-181.
14. Riezzo G, Chiloiri M, Guerra V. Electrogastrography in healthy children: evaluation of normal values, influence of age, gender, and obesity. *Dig Dis Sci* 1998;43:1646-1651.
15. Stein PK, Bosner MS, Kleiger RE, Conger BM. Heart rate variability: a measure of cardiac autonomic tone. *Am Heart J* 1994;127:1376-1381.
16. Sztajzel J. Heart rate variability: a noninvasive electrocardiographic method to measure the autonomic nervous system. *Swiss Med Wkly* 2004;134:514-522.
17. van Ravenswaaij-Arts CM, Koll e LA, Hopman JC, Stoeltinga GB, van Geijn HP. Heart rate variability. *Ann Intern Med* 1993;118:436-447.
18. Qin C, Chen JD, Zhang J, Foreman RD. Characterization of T9-T10 spinal neurons with duodenal input and modulation by gastric electrical stimulation in rats. *Brain Res* 2007;1152:75-86.
19. Qin C, Chen JD, Zhang J, Foreman RD. Modulatory effects and afferent pathways of gastric electrical stimulation on rat thoracic spinal neurons receiving input from the stomach. *Neurosci Res* 2007;57:29-39.
20. Qin C, Sun Y, Chen JD, Foreman RD. Gastric electrical stimulation modulates neuronal activity in nucleus tractus solitarii in rats. *Auton Neurosci* 2005;119:1-8.
21. Sun X, Tang M, Zhang J, Chen JD. Excitatory effects of gastric electrical stimulation on gastric distension responsive neurons in ventromedial hypothalamus (VMH) in rats. *Neurosci Res* 2006;55:451-457.
22. Sun Y, Qin C, Foreman RD, Chen JD. Intestinal electric stimulation modulates neuronal activity in the nucleus of the solitary tract in rats. *Neurosci Lett* 2005;385:64-69.
23. Tang M, Zhang J, Chen JD. Central mechanisms of gastric electrical stimulation involving neurons in the paraventricular nucleus of the hypothalamus in rats. *Obes Surg* 2006;16:344-352.
24. Chen JD, Schirmer BD, McCallum RW. Serosal and cutaneous recordings of gastric myoelectrical activity in patients with gastroparesis. *Am J Physiol* 1994;266(1 Pt 1):G90-G98.
25. Lu CL, Montgomery P, Zou X, Orr WC, Chen JD. Gastric myoelectrical activity in patients with cervical spinal cord injury. *Am J Gastroenterol* 1998;93:2391-2396.
26. Lu CL, Shan DE, Chen CY, et al. Impaired gastric myoelectrical activity in patients with Parkinson's disease and effect of levodopa treatment. *Dig Dis Sci* 2004;49:744-749.
27. Lu CL, Zou X, Orr WC, Chen JD. Postprandial changes of sympathovagal balance measured by heart rate variability. *Dig Dis Sci* 1999;44:857-861.
28. Kr uger C, Kalenka A, Haunstetter A, et al. Baroreflex sensitivity and heart rate variability in conscious rats with myocardial infarction. *Am J Physiol* 1997;273(5 Pt 2):2240-2247.
29. Paxinos G, Watson C. *The rat brain in stereotaxic coordinates*. 5rd ed. San Diego: Academic Press 2005.
30. Cigaina V. Gastric pacing as therapy for morbid obesity: preliminary results. *Obes Surg* 2002;12(suppl 1):12S-16S.
31. Cigaina V. Long-term follow-up of gastric stimulation for obesity: the Mestre 8-year experience. *Obes Surg* 2004;14(suppl 1):S14-S22.
32. Wang GJ, Yang J, Volkow ND, et al. Gastric stimulation in obese subjects activates the hippocampus and other regions involved in brain reward circuitry. *Proc Natl Acad Sci USA* 2006;103:15641-15645.
33. Mercer JG, Archer ZA. Diet-induced obesity in the Sprague-Dawley rat: dietary manipulations and their effect on hypothalamic neuropeptide energy balance systems. *Biochem Soc Trans* 2005;33(Pt 5):1068-1072.
34. Buettner R, Sch olmerich J, Bollheimer LC. High-fat diets: modeling the metabolic disorders of human obesity in rodents. *Obesity (Silver Spring)* 2007;15:798-808.
35. Peckham SC, Entenman C. The influence of a hypercaloric diet on gross body and adipose tissue composition in the rat. *Res Dev Tech Rep* 1962;23:187-197.
36. Chang S, Graham B, Yakubu F, Lin D, Peters JC, Hill JO. Metabolic differences between obesity-prone and obesity-resistant rats. *Am J Physiol* 1990;259(6 Pt 2):R1103-R1110.
37. Gao J, Ghibaudi L, van Heek M, Hwa JJ. Characterization of diet-induced obese rats that develop persistent obesity after 6 months of high-fat followed by 1 month of low-fat diet. *Brain Res* 2002;936:87-90.
38. Levin BE, Dunn-Meynell AA, Balkan B, Keesey RE. Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. *Am J Physiol* 1997;273(2 Pt 2):R725-R730.
39. Klein S, Luu K, Gasic S, Green A. Effect of weight loss on whole body and cellular lipid metabolism in severely obese humans. *Am J Physiol* 1996;270(5 Pt 1):E739-E745.
40. Dobrian AD, Davies MJ, Prewitt RL, Lauterio TJ. Development of hypertension in a rat model of diet-induced obesity. *Hypertension* 2000;35:1009-1015.
41. Levin BE, Dunn-Meynell AA, Ricci MR, Cummings DE. Abnormalities of leptin and ghrelin regulation in obesity-prone juvenile rats. *Am J Physiol Endocrinol Metab* 2003;285:E949-E957.
42. Teske JA, Levine AS, Kuskowski M, Levine JA, Kotz CM. Elevated hypothalamic orexin signaling, sensitivity to orexin A, and sponta-

- neous physical activity in obesity-resistant rats. *Am J Physiol Regul Integr Comp Physiol* 2006;291:R889-R899.
43. Vrang N, Madsen AN, Tang-Christensen M, Hansen G, Larsen PJ. PYY(3-36) reduces food intake and body weight and improves insulin sensitivity in rodent models of diet-induced obesity. *Am J Physiol Regul Integr Comp Physiol* 2006;291:R367-R375.
  44. Green GM, Guan D, Schwartz JG, Phillips WT. Accelerated gastric emptying of glucose in Zucker type 2 diabetic rats: role in postprandial hyperglycaemia. *Diabetologia* 1997;40:136-142.
  45. Park MI, Camilleri M. Gastric motor and sensory functions in obesity. *Obes Res* 2005;13:491-500.
  46. Dubois A. Obesity and gastric emptying. *Gastroenterology* 1983;84:875-876.
  47. Horowitz M, Collins PJ, Cook DJ, Harding PE, Shearman DJ. Abnormalities of gastric emptying in obese patients. *Int J Obes* 1983;7:415-421.
  48. Tosetti C, Corinaldesi R, Stanghellini V, et al. Gastric emptying of solids in morbid obesity. *Int J Obes Relat Metab Disord* 1996;20:200-205.
  49. Wisén O, Johansson C. Gastrointestinal function in obesity: motility, secretion, and absorption following a liquid test meal. *Metabolism* 1992;41:390-395.
  50. Wright RA, Krinsky S, Fleeman C, Trujillo J, Teague E. Gastric emptying and obesity. *Gastroenterology* 1983;84:747-751.
  51. Cardoso-Júnior A, Coelho LG, Savassi-Rocha PR, et al. Gastric emptying of solids and semi-solids in morbidly obese and non-obese subjects: an assessment using the <sup>13</sup>C-octanoic acid and <sup>13</sup>C-acetic acid breath tests. *Obes Surg* 2007;17:236-241.
  52. Jackson SJ, Leahy FE, McGowan AA, Bluck LJ, Coward WA, Jebb SA. Delayed gastric emptying in the obese: an assessment using the non-invasive <sup>13</sup>C-octanoic acid breath test. *Diabetes Obes Metab* 2004;6:264-270.
  53. Verdich C, Madsen JL, Toubro S, Buemann B, Holst JJ, Astrup A. Effect of obesity and major weight reduction on gastric emptying. *Int J Obes Relat Metab Disord* 2000;24:899-905.
  54. Park MI, Camilleri M, O'Connor H, et al. Effect of different macronutrients in excess on gastric sensory and motor functions and appetite in normal-weight, overweight, and obese humans. *Am J Clin Nutr* 2007;85:411-418.
  55. Levin BE. Sympathetic activity, age, sucrose preference, and diet-induced obesity. *Obes Res* 1993;1:281-287.
  56. Levin BE. Defective cerebral glucose utilization in diet-induced obese rats. *Am J Physiol* 1991;261(4 Pt 2):R787-R792.
  57. Levin BE, Sullivan AC. Differences in saccharin-induced cerebral glucose utilization between obesity-prone and -resistant rats. *Brain Res* 1989;488:221-232.
  58. Levin BE, Triscari J, Hogan S, Sullivan AC. Resistance to diet-induced obesity: food intake, pancreatic sympathetic tone, and insulin. *Am J Physiol* 1987;252(3 Pt 2):R471-R478.
  59. Levin BE, Triscari J, Sullivan AC. Altered sympathetic activity during development of diet-induced obesity in rat. *Am J Physiol* 1983;244:R347-R355.
  60. Bray GA. Obesity, a disorder of nutrient partitioning: the MONA LISA hypothesis. *J Nutr* 1991;121:1146-1162.
  61. Nijima A, Rohner-Jeanrenaud F, Jeanrenaud B. Role of ventromedial hypothalamus on sympathetic efferents of brown adipose tissue. *Am J Physiol* 1984;247(4 Pt 2):R650-R654.
  62. Sakaguchi T, Arase K, Bray GA. Sympathetic activity and food intake of rats with ventromedial hypothalamic lesions. *Int J Obes* 1988;12:285-291.
  63. Vander Tuig JG, Knehans AW, Romsos DR. Reduced sympathetic nervous system activity in rats with ventromedial hypothalamic lesions. *Life Sci* 1982;30:913-920.
  64. Young JB, Landsberg L. Impaired suppression of sympathetic activity during fasting in the gold thioglucose-treated mouse. *J Clin Invest* 1980;65:1086-1094.
  65. Tentolouris N, Liatis S, Katsilambros N. Sympathetic system activity in obesity and metabolic syndrome. *Ann N Y Acad Sci* 2006;1083:129-152.
  66. Liatis S, Tentolouris N, Katsilambros N. Cardiac autonomic nervous system activity in obesity. *Pediatr Endocrinol Rev* 2004;1(suppl 3):476-483.
  67. Young JB, Macdonald IA. Sympathoadrenal activity in human obesity: heterogeneity of findings since 1980. *Int J Obes Relat Metab Disord* 1992;16:959-967.
  68. Laederach-Hofmann K, Mussgay L, Rüdell H. Autonomic cardiovascular regulation in obesity. *J Endocrinol* 2000;164:59-66.
  69. Muscelli E, Emdin M, Natali A, et al. Autonomic and hemodynamic responses to insulin in lean and obese humans. *J Clin Endocrinol Metab* 1998;83:2084-2090.
  70. Bouret SG, Gorski JN, Patterson CM, Chen S, Levin BE, Simerly RB. Hypothalamic neural projections are permanently disrupted in diet-induced obese rats. *Cell Metab* 2008;7:179-185.
  71. Gorski JN, Dunn-Meynell AA, Levin BE. Maternal obesity increases hypothalamic leptin receptor expression and sensitivity in juvenile obesity-prone rats. *Am J Physiol Regul Integr Comp Physiol* 2007;292:R1782-R1791.
  72. Levin BE, Dunn-Meynell AA. Reduced central leptin sensitivity in rats with diet-induced obesity. *Am J Physiol Regul Integr Comp Physiol* 2002;283:R941-R948.
  73. Levin BE, Dunn-Meynell AA, Banks WA. Obesity-prone rats have normal blood-brain barrier transport but defective central leptin signaling before obesity onset. *Am J Physiol Regul Integr Comp Physiol* 2004;286:R143-R150.
  74. Levin BE, Dunn-Meynell AA. Dysregulation of arcuate nucleus preproneuropeptide Y mRNA in diet-induced obese rats. *Am J Physiol* 1997;272(5 Pt 2):R1365-R1370.
  75. Hellström PM, Geliebter A, Näslund E, et al. Peripheral and central signals in the control of eating in normal, obese and binge-eating human subjects. *Br J Nutr* 2004;92(suppl 1):S47-S57.
  76. Geraciotti TD, Loosen PT, Ebert MH, Schmidt D, Ekthator NN. Fasting and postprandial cerebrospinal fluid glucose concentrations in healthy women and in an obese binge eater. *Int J Eat Disord* 1995;18:365-369.